REMARKS

This Amendment is respectfully submitted in response to the Office Action dated July 17, 2006. Claims 6, 23-25, 31-35, 39-44, 46, 48-52, 54-56, 58-64, 66, 67 and 69-77 are pending in the application. Claims 24, 28-30, 32, 36-38, 45, 47-48, 52-53, 57, 65, 68 and 71-80 have been cancelled without prejudice or disclaimer. Claims 6, 23, 31, 39, 44, 46, 51 and 64 have been amended. Claims 81 and 82 have been added. No new subject matter has been added by the amendments or new claims.A Petition for a three-month extension of time and a supplemental information disclosure statement are submitted herewith. The Commissioner is hereby authorized to charge deposit account 02-1818 for any fees which are due and owing.

In the Office Action, Claims 6, 23-25, 31-35, 39-44, 46, 48-52, 54-56, 58-64, 66, 67 and 69-77 are rejected under 35 U.S.C. §112, first paragraph, for allegedly including subject matter not sufficiently described in the Specification. Applicants traverse this rejection and respectfully submit that the rejection has been overcome for the reasons set forth below.

According to the Office Action, a "retinoid receptor," as recited in Claims 49, 59 and 69 is not the same as a "retinoic acid receptor," and that reciting a retinoid receptor "constitutes new matter." Office Action, page 2. Not only is a retinoid receptor recited in original Claims 2 and 13, the Specification also clearly indicates at, for example,

> In a preferred embodiment of the method, the c-myc gene is fused with other DNA elements, where the other DNA elements comprise at least one element selected from the group consisting of a ligand binding domain for an estrogen receptor, an androgen receptor, a progesterone receptor, a glucocorticoid receptor, a thyroid hormone receptor, a retinoid receptor, and an ecdysone receptor.

Therefore, Claims 49, 59 and 69 do not add new matter and are in condition for allowance.

According to the Office Action, the reference in the preamble of independent Claims 51 and 64 to a "portion" of the cell line has no antecedent basis. Applicants note that page 19 of the Specification refers to "proportion" instead of "portion" and have amended Claims 51 and 64 to refer to a proportion of the cell line capable of differentiating into neurons.

The Office Action also alleges that independent Claims 6, 23, 31, 51, 64 and 72 lack support for "resists differentiation in media containing a mitogen." As set forth in MPEP

§2163.02, the subject matter of the claim need not be described literally (i.e., using the same terms or *in haec verba*) in order for the disclosure to satisfy the description requirement. Applicants have made clear throughout the Specification that the inventions are directed to cell line (Claim 6 and 51) neural precursor cells (Claim 23, 31 and 72) and modified cell (Claim 64) that resist(s) differentiation in media containing a mitogen. For example, the last paragraph on page 10 of the Specification sets forth the following:

> Human and rat CNS stem cells harboring the fusion gene were grown in the continuous presence of mitogens and β-estradiol in the culture medium. Growth of the cells were significantly more robust, exhibiting faster mitotic rate, resistance to spontaneous differentiation, and much greater overall stability during the expansion.

Therefore, Applicants respectfully submit that there is adequate written description for this claimed feature.

The Office Action contends that reference to "receptor ligand-regulated c-myc gene" as recited in Claims 6, 23, 31 and 51, "nuclear receptor" as recited in Claims 48, 51 and 64 and "a c-myc protein fused with at least one nuclear receptor" in Claim 64 constitutes new matter. Office Action, page 3. In addition, the Office Action contends that there is lack of support for "c-myc-activating agent" as recited in Claims 31, 51, 64 and 71.

The Specification provides at, for example, page 25 and 26, a description of a ligand binding mechanism common to members of the nuclear receptor superfamily. The Specification further emphasizes alternatives to the estrogen receptor and β-estradiol system:

> The ligand binding domain of these nuclear receptor proteins and their ligands can substitute for the estrogen receptor and β-estradiol in order to regulate functions of the fused c-myc protein moiety.

The Specification goes on to list a sufficient variety of species of nuclear receptors other than the estrogen receptor to reflect the variation within the nuclear receptor superfamily genus necessary to support claims to the genus. The operability of members of the nuclear receptor superfamily in the invention is predictable by one of skill in the art. *In re Curtis*, 354 F.3d 1347, 1358, 69 USPQ2d 1274, 1282 (Fed. Cir. 2004). MPEP §2163.02. Accordingly, Applicants have

amended Claims 6, 23 and 31 to refer to a "nuclear receptor". Applicants have also amended Claims 31 and 51 to provide a *c-myc*-activating agent capable of binding to the ligand-binding domain of a nuclear receptor. In addition, Applicants have amended Claims 64 and 71 to include a nuclear receptor ligand. Therefore, Applicants respectfully submit that the Specification provides adequate support for these features as amended.

The Office Action suggests that there is no support for "wherein the second mitogen is different from the first mitogen" as recited in Claims 40 and 58. Not only do original Claims 1 and 12 disclose the second mitogen being other than the first mitogen, the Specification at, for example, pages 4 and 5 also discloses this feature. Therefore, Applicants respectfully submit that the Specification provides adequate support for this feature as amended.

The Office Action states that there is no support for a monolayer component as recited in Claim 39. Applicants have amended Claim 39 to recite a feeder cell component in accordance with the recommendations in the Office Action.

The Office Action also states that there is no support for a clonal cell line as recited in Claims 44, 46 and 73. However, the Specification specifically refers to clonal cell lines throughout the description. For example, the Specification at pages 2-3, 5 and 10 provides the following:

> The culture condition permits nearly pure populations of CNS stem cells for a long period both as a mass culture and as a clonal culture.
>
> The present application also reveals a method for producing stable clonal cell lines of mammalian neural precursor cells in vitro.
>
> Here, a reproducible and efficient method utilizing over-expression of the c-myc gene to stabilize the differentiation potentials of neural cells and to isolate stable clonal cell lines is described. Specification, page 10.

Furthermore, the Specification provides a description of the initiation of a clonal cell line through the isolation of clones. Specification, page 13 et seq. Therefore, Applicants respectfully submit that the Specification provides sufficient support for "a clonal cell line" as recited in Claims 44, 46 and 73.

The Office Action further states that there is no written description of the "c-myc gene". Not only is the c-myc gene disclosed in original Claims 1, 2, 12 and 13, the Specification also refers to the c-myc gene throughout the disclosure such as pages 4, 6, 7, 10, 11, etc. The Office Action seems to suggest that the sequence of the c-myc gene must be disclosed in the specification when the Office Action refers to the c-myc gene being "structurally definable" by its 5' and 3' flanking regions. Office Action, page 4. The claimed invention is not directed to the c-myc gene itself. As set forth in the Specification at, for example, page 24, c-myc has been previously used to transform cells of non-neural origins. Information that is well known in the art, such as the c-myc gene, need not be described in detail in the specification to meet the written description requirement. See, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986). Therefore, Applicants respectfully submit that there is adequate written description to support claims including the c-myc gene.

In the Office Action, Claim 77 was rejected under 35 U.S.C. §112, second paragraph, for allegedly being indefinite. Applicants have canceled Claim 77 without prejudice or disclaimer and respectfully submit that the rejection has been overcome.

Accordingly, for at least the reasons set forth above, Applicants respectfully submit that the rejections under 35 U.S.C. §112, first paragraph, have been overcome or are improper and should be withdrawn.

In the Office Action, Claims 23-25, 31-35, 39-44, 46, 48-52, 54-56, 58-64, 66, 67 and 69-77 are rejected under 35 U.S.C. §102(b) as being anticipated by *Nakafuku* et al. (" *Nakafuku*"). Applicants gratefully acknowledge that the rejection of Claim 6 under 35 U.S.C. §102(b) as being anticipated by *Nakafuku* has been withdrawn as stated in the Office Action on page 1. Also, Claims 6, 23, 25, 31, 33 to 35, 39 to 44, 46, 48 to 51, 54 to 64, 66, 67 and 69 to 77 are rejected under 35 U.S.C. §103(a) as being unpatentable over *Nakafuku* in view of the publication to *Eilers* et al. ("*Eilers*") and/or the publication to *Evans* et al. ("*Evans*"). Applicants respectfully traverse the rejection of the remaining claims for at least the reasons discussed below and submit that the rejections have been overcome.

In response, Applicants have amended the claims to include an amount of nuclear receptor ligand sufficient to maintain a stable cell line. Support for this amendment can be found

at, for example, page 25 of the Specification and the example provided at page 13 of the Specification. Therefore, Applicants respectfully submit that no new matter is added by this amendment.

As acknowledged in the Office Action, *Nakafuku*, *Eilers* and *Evans* do not teach a stable culture of human neural progenitor cells as provided in each of the independent claims. The Office Action alleges, however, that it would have been obvious to one of ordinary skill in the art to combine the techniques taught by *Nakafuku*, *Eilers* and *Evans* to be applied in rat cells to produce stable human neural precursor cells or cell lines.

Nakafuku, *Eilers* and *Evans* do not disclose producing stable mammalian neural precursor cells. Specifically, *Nakafuku* either alone or in combination with *Eilers* and *Evans* does not disclose an amount of nuclear receptor ligand sufficient to maintain a stable cell line as recited in each of the amended independent claims. *Nakafuku*, for example, does not disclose culturing the cells in the presence of an amount of β-estradiol sufficient to stabilize the cells. *Nakafuku* discloses adding different combinations of growth factor and β-estradiol to maximize cell division and differentiation without regard to stabilizing cell growth. In fact, the cells in *Nakafuku* are stimulated to progress through each stage of the cell cycle as rapidly as possible proliferating until they differentiate. There is no discussion in *Nakafuku* of preventing differentiation and transformation of the cells based on the amount of β-estradiol added to the culture media in order to stabilize the cells. Neither *Eilers* nor *Evans* cure this deficiency in *Nakafuku*. Not only do the references not suggest that these techniques can be used to produce stable human neural precursor cells, there is nothing in the references to suggest that these techniques can be used to produce stable mammalian neural precursor cells or cell lines.

Furthermore, as set forth in the Declaration of Dr. Karl Johe Under 37 C.F.R. §1.132, attached hereto at Exhibit A, because of the differences in functional characteristics of human cells and rat cells, one of skill in the art would not have expected the techniques employed in rat cells to be successful in human cells. Therefore, one of skill in the art would not have been motivated by the references or the knowledge in the art to combine the references to produce a stable cell line.

Accordingly, for at least the reasons provided above, Applicants respectfully submit that the rejections have been overcome and the claims are in condition for allowance.

An earnest endeavor has been made to place this application in condition for allowance and such allowance is courteously solicited. If the Examiner has any questions related to this Response, Applicants respectfully submit that the Examiner contact the undersigned. Respectfully submitted,

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BY 

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Dated: January 17, 2007

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Renji Yang et al.
Appl. No.: 10/047,352
Conf. No.: 4868
Filed: January 14, 2002
Title: STABLE NEURAL STEM CELL LINES
Art Unit: 1649
Examiner: Robert Clinton Hayes
Docket No.: 109015-024

DECLARATION OF DR. KARL K. JOHE UNDER 37 C.F.R. § 1.132

I, Karl K. Johe, hereby declare and state as follows:

1. My education and professional experience are attached with my curriculum vitae as Exhibit A.

2. I am one of the inventors of the above-referenced U.S. Patent Application Serial Number 10/047,352 entitled, "STABLE NEURAL STEM CELL LINES".

3. I understand that this declaration is being submitted in connection with an Office Action dated July 17, 2006, ("the Office Action"), and I have reviewed the Office Action.

4. It was well known at the time of this invention that human neural stem cells are functionally distinct from rat neural stem cells.

5. Human neural stem cells have different growth requirements than rat neural stem cells. For example, Vescovi et al (Exp. Neurol., 1999 Mar; 156(1): 71-83 attached hereto) states:

> "We show that the developing human CNS embodies multipotent precursors that differ from their murine counterpart in that they require simultaneous, synergistic stimulation by both epidermal and fibrobast growth factor-2 to exhibit critical stem cell characteristics." (Abstract).

6. Human neural stem cells have different growth characteristics than rat neural stem cells. For example, as we reported on page 15 of the Specification of this application, under identical culture conditions, doubling time of human cells (36-60 hours) is much longer than that of rat cells (18-24 hours). Also, the overall mitotic capacity of the human cells (20-30 cell doublings) is much greater than that of rat cells (12-16 cell doublings).

7. The differences between human neural stem cells and rat neural stem cells are demonstrated in the different requirements for cell proliferation taught by Nakafuku et al. (Establishment and Characterization of a Multipotenital Neural Cell Line That Can Conditionally Generate Neurons, Astrocytes, and Oligodendrocytes; Joural of Neuroscience Research 41:153-168 (1995) ("*Nakafuku*")). The cells of rat multipotential neural precursor lines in *Nakafuku* require simultaneous stimulation by both c-myc and bFGF for maximal differentiation of MNS-57 cell to occur and to be committed to generating multiple neural cell types. See Nakafuku, Abstract and paragraph 3 of page 166. In contrast, human neural stem cells, as we reported in the Specification at, for example, the last sentence on page 10, neither bFGF nor c-myc activation for differentiation or commitment for multiple lineage. In fact, we report that mitogen must be withdrawn to initiate differentiation of human neural stem cells.

8. The differences between human neural stem cells and rat neural stem cells are demonstrated in the different patterns of differentiation taught by *Nakafuku*. In *Nakafuku*, only 0.3% of rat cells grown in monolayer culture differentiate into MAP2-positive neurons. See, for example pg. 162, TABLE I. In contrast 20% or more of the human cells differentiate into MAP2-positive neurons as we reported at, for example, on page 19 of the Specification.

9. Therefore, based on these differences between rat neural stem cells and human neural stem cells, I would not have expected techniques disclosed in Evans or Eiler's applied to rat cells disclosed by Nakafuka to have the same effect in human neural stem cells as suggested in the Office Action.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this patent and any patent issuing therefrom.

FURTHER DECLARANT SAYETH NOT:

Karl K. Johe, Ph.D.

Signed at Rockville, Maryland This 17th day of January, 2007.